Nanostructured emulsions and nanolaminates for delivery of active ingredients: Improving food safety and functionality

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1 ABSTRACT

Background

Nowadays, consumers are increasingly demanding high-quality, safe and healthy food products. Nanostructured emulsions and nanolaminates may have the potential to protect and transport lipophilic and hydrophilic active compounds commonly incorporated to food products, such as natural antimicrobials and nutraceuticals, while protecting or even enhancing their functional properties.

Scope and approach

This review deals with the most important aspects concerning to the use of nanostructured emulsions and nanolaminates as delivery systems of active ingredients, including the advantages and challenges of incorporating plant-derived antimicrobials and nutraceuticals in foods, relevant factors affecting the formation of these nanostructures, fabrication methods, their advantages as delivery systems, and the current trends in food applications. In addition, concerns regarding the potential toxicity of nanomaterials are also discussed.

Key findings and conclusions

The successful production of nanostructured emulsions and nanolaminates depends on several physicochemical factors that should be controlled in order to reach stable systems. Research evidences that nanostructured emulsions and nanolaminates are able to improve the delivery and biological activity of encapsulated active compounds. Antimicrobial and bioactive nanostructured emulsions and nanolaminates exhibit some promising advantages in food preservation and may represent a new strategy to produce functional foods. However, the knowledge in this area is still limited. The potential toxicological effects of nanostructured delivery systems are a current concern. Therefore, future investigations should be directed towards more comprehensive studies to shed light on the formation, physicochemical stability, functional performance, interactions with food matrices and toxicity of nanostructured delivery systems before their commercialization.

Keywords

Nanoemulsions, multilayer emulsions, nanolaminates, delivery systems, essential oils, nutraceuticals

1. Introduction

The increasing consumer's demand of fresh-like food products and the rejection of synthetic additives are driving the scientific community to pursue natural alternatives that can enhance food preservation while having a minimum effect on the organoleptic and nutritional attributes of the product. Moreover, consumption patterns are changing toward a healthy diet owing to an evident relationship between food and health. As a result, there is a global trend towards the intake of food products with health-promoting properties beyond their nutritional value. There are a number of antimicrobials and nutraceuticals from natural sources with great performance that allow reducing or even replacing the use of their synthetic counterparts in foods (Irkin & Esmer, 2015; Oliveira, Ramos, Ramos, Piccoli, & Cristianini, 2015). However, an effective incorporation of active compounds to foods may be restricted by their physicochemical properties, stability under certain conditions or low bioavailability. As a result, there is a need of encapsulating them into delivery systems, understood as those in which an active compound is entrapped into a carrier (Fathi, Mozafari, & Mohebbi, 2012) , that allow overcoming these issues.

Nanotechnology is offering innumerable approaches in the food field (Cushen, Kerry, Morris, Cruz-Romero, & Cummins, 2012; Durán & Marcato, 2013). Nanostructured delivery systems constitute one of the most explored approaches. Their nanostructured architecture enables to improve protection of encapsulated compounds, increase solubility and dispensability of lipophilic ingredients in water-based environments, modulate the compound release, or even increase bioavailability of nutraceuticals, exhibiting better performance than systems of bigger particle sizes (Augustin & Hemar, 2009; Ezhilarasi, Karthik, Chhanwal, & Anandharamakrishnan, 2013). In particular, emulsion-based delivery systems of either one single layer, such as nanoemulsions, or multiple layers have been proposed as those capable of effectively encapsulating lipophilic active compounds (Augustin & Hemar, 2009). On the other hand, nanolaminates are systems that can be applied directly onto food surfaces as an edible coating or to functionalize the surface of conventional packaging. The most important advantage is their ability of serving as reservoirs of active compounds, either hydrophilic or

lipophilic, protecting them and modulating its release in response to certain triggers (Kuan, Yee-Fung, Yuen, & Liong, 2012; Rojas-Graü, Soliva-Fortuny, & Martín-Belloso, 2009). The use of nanostructured emulsions and nanolaminates for delivery of active ingredients to foods represents a promising alternative to improve the quality, safety and functionality of food products. In this review we discuss the properties and limitations of incorporating plant-based antimicrobials and nutraceuticals in foods, and overview the recent developments concerning the formation, physicochemical characteristics, fabrication techniques, advantages as delivery systems, and food applications of a selected number of nanostructured emulsions and nanolaminates (Fig 1). Finally, the toxicological aspects associated to the incorporation of nanomaterials in foods are presented.

2 ACTIVE INGREDIENTS: ADVANTAGES AND LIMITATIONS OF THEIR INCORPORATION IN FOODS

2.1 PLANT-BASED ANTIMICROBIALS

Antimicrobial compounds that come from plant sources exhibit outstanding efficacy against most pathogenic microorganisms responsible of foodborne illnesses and food spoilage (Tiwari et al., 2009). There is a strong consumer's perception that natural preservatives have less side effects to health that their non-natural counterparts, although in some cases the concentration required to achieve an antimicrobial effect is greater than that needed with synthetic preservatives (Carocho, Barreiro, Morales, & Ferreira, 2014). Antimicrobials derived from plants are substances originated from their secondary metabolism, which plays a protective role against predators or stressing conditions (Solórzano-Santos & Miranda-Novales, 2012). Table 1 summarizes some types of plant-based antimicrobials commonly used in foods. Essential oils (EOs), the most significant group of plant-based antimicrobials, are complex mixtures of volatile compounds present in many herbs and spices (Burt, 2004). The main groups of compounds responsible for their antimicrobial and antioxidant properties include phenolic acids, quinones, saponins, flavonoids, tannins, coumarins, terpenoids and alkaloids (Bassolé & Juliani, 2012; Lai & Roy, 2004).

Despite the increasing interest in applying EOs for food preservation, there are several factors affecting their antimicrobial activity, such as the poor-water solubility, partitioning behavior, mass transfer, volatility or reactivity can influence its efficacy in food systems (Donsì & Ferrari, 2016; Prakash, Kedia, Mishra, & Dubey, 2015). In addition, the use of EOs significantly changes the organoleptic profile of foods or may be toxic at high concentrations (Dima & Dima, 2015). Antimicrobial efficacy of EOs may be also influenced by the pH, fat content or water activity present in the food matrix. Plant-derived antimicrobials may bind to lipids, proteins or carbohydrates in foodstuffs, requiring higher concentrations than those used in *in vitro* studies to achieve the same effect (Weiss, Loeffler, & Terjung, 2015).

2.2 NUTRACEUTICALS

There are several nutraceuticals that can be incorporated into food formulations with the purpose of providing well-being while reducing the incidence of diseases in humans. Table 1 presents some of the nutraceutical compounds that could be potentially included in foods. The intake of recommended doses of these compounds has been associated with prevention of coronary heart disease, diabetes, obesity, hypertension, and cancer (Cencic & Chingwaru, 2010; Espín, García-Conesa, & Tomás-Barberán, 2007). Being isolated from natural sources, nutraceuticals are expected to exhibit relatively less toxicity and less secondary side effects than drugs used to treat similar symptoms (Ting, Jiang, Ho, & Huang, 2014).

However, effective enrichment and fortification of food products using nutraceuticals represents a major challenge. The chemical stability of most bioactive compounds is highly influenced by pH, temperature, oxygen, light or specific chemicals that promote the loss of the biological properties. Moreover, the oral bioavailability of nutraceuticals depends on their solubility in the gastrointestinal tract, stability during digestion and intestinal permeability (Gleeson, Ryan, & Brayden, 2016). Therefore, nutraceuticals may have poor oral bioavailability as a result of several physicochemical and physiological processes occurring after intake in the gastrointestinal tract.

3 NANOSTRUCTURED DELIVERY SYSTEMS

3.1 NANOEMULSIONS

Nanoemulsions are oil-in-water systems containing oil droplets with mean diameters between 20 nm and 200 nm (Solans, Izquierdo, Nolla, Azemar, & Garcia-Celma, 2005). In an emulsion, two immiscible liquids (e.g. oil and water) are combined so that one liquid (disperse phase) is incorporated as droplets within a second liquid (continuous phase). These two phases are often joint by a process known as homogenization, in which the use of energy is required for increasing the surface area of the disperse phase and create droplets. As the total free energy of formation is always positive and the interfacial tension between both phases is large, emulsions are thermodynamically unstable (Tadros, Izquierdo, Esquena, & Solans, 2004). Nanoemulsions are also thermodynamically unstable but the rate of destabilization is lower than in conventional emulsions because the Brownian motion effect is sufficient to overcome the gravitational forces (Mason, Wilking, Meleson, Chang, & Graves, 2006; Tadros et al., 2004). For this reason, nanoemulsions are more stable to sedimentation, creaming, flocculation and coalescence than conventional emulsions. There are some important differences in terms of physicochemical properties, such as the fact that nanoemulsions are less opaque than conventional emulsions, since droplet diameters are smaller than the wavelength of light, and hence, droplets scatter light weakly (McClements, 2011). This feature makes nanoemulsions suitable systems to be incorporated into food products such as beverage, sauces, dressing or soups, having a little impact on their sensorial properties.

3.1.1 Factors affecting the formation and fabrication methods

The formulation of oil-in-water nanoemulsions is a key factor that determines their overall properties including droplet size, interfacial properties, physical stability or their functionality (Salvia-Trujillo, Rojas-Graü, Soliva-Fortuny, & Martín-Belloso, 2014). The physicochemical characteristics of the oil phase have an important impact on the formation and stability of nanoemulsions. For instance, when viscosity of the oil phase is high, droplets disruption is more difficult by mechanical means (Jafari, Assadpoor, He, & Bhandari, 2008). The viscosity ratio between oil phase and aqueous phase also

affects the final droplet size achieved. The closer the viscosity ratio between the oil and aqueous phase is to the unit, the more efficient the homogenization and the smaller the droplet size in nanoemulsions (Qian & McClements, 2011). The oil solubility also plays an important role in nanoemulsions stability. Oils with considerable water-solubility give rise to Ostwald ripening destabilization phenomenon, which consists in the formation of bigger droplets fueled by small ones driven by their fast diffusion along the continuous phase of nanoemulsions (Wooster, Golding, & Sanguansri, 2008). Many essential oils have significant water-solubility and Ostwald ripening has been described as the most frequent reason of destabilization of nanoemulsions containing them (Guerra-Rosas, Morales-Castro, Ochoa-Martínez, Salvia-Trujillo, & Martín-Belloso, 2016). The surfactant type and concentration have been found to exhibit a great impact in the formation of nanoemulsions. Each surfactant acts differently at the oil-water interface of emulsions depending on their molecular structure, which determines their hydrophilic-lipophilic balance (HLB) and their ability to adsorb to the oil-water interface and reduce the interfacial tension. The droplet size of nanoemulsions normally decreases as the surfactant concentration increases (Silva, Cerqueira, & Vicente, 2015). Small molecule surfactants (Tweens, sucrose esters, spans, SDS) are able to generate nano-sized emulsions (Silva, Cerqueira, & Vicente, 2011). However, there is a marked trend in using natural-derived surface-active molecules in nanoemulsions to develop “label-friendly” food products. In this case, proteins, polysaccharides or phospholipids, have been reported as feasible alternatives (Bai, Huan, Gu, & McClements, 2016; Gupta & Ghosh, 2015). Another important component in nanoemulsions formulation is the presence of texturizing agents in the aqueous phase. The incorporation of biopolymers, such as polysaccharides or proteins, at sufficient concentrations modifies the viscosity and improve emulsion stability, by delaying collision between droplets (McClements & Rao, 2011), as well as to provide specific textural properties, such as the film-forming ability.

There are two main approaches commonly used to produce fine emulsions. The high-energy methods (Mason et al., 2006) and low-energy methods (Conxita Solans & Solé, 2012). High energy methods are based in the input of large amounts of energy to reach emulsification. To prepare nanoemulsions,

preliminary coarse emulsions are normally obtained by high shear mixing, which are further subjected to a second homogenization. High pressure homogenization or sonication are the most commonly known techniques to produce nanoemulsions, which involve the use of mechanical forces, able to create intensive disruptive forces such as, turbulence, shear or cavitation, that break down the droplets. The smallest droplet size that can be obtained by high energy methods depends on the type of homogenizer, operating conditions, emulsion composition, and the physicochemical characteristics of the emulsion components (Mason et al., 2006). The low-energy methods consists on the spontaneous emulsification by controlling the physicochemical properties of the system. Self-emulsification and phase inversion have been described as reliable techniques to fabricate nanoemulsions. Both use the internal chemical energy generated in the system, either by the dilution process with the continuous phase, or by the phase transitions taking place during emulsification, to produce nano-sized droplets (Conxita Solans & Solé, 2012).

3.1.2 Advantages as delivery systems

These systems are able to encapsulate lipophilic active compounds within the oil phase of nanoemulsions. A reduction in the droplet size down to the nanoscale increases the surface area per volume unit of the disperse phase, having important impact on the physicochemical characteristics and functionality of nanoemulsions (Gupta, Eral, Hatton, & Doyle, 2016). The fact that nanoemulsions are translucent systems represents a major advantage, since they may have little impact on the visual properties of the food product, while contributing to enhance other food characteristics. In fact, this feature has been already observed in nanoemulsions containing some types of EOs which, beyond their antimicrobial effect, are often more translucent than conventional emulsions (Salvia-Trujillo, Rojas-Graü, Soliva-Fortuny, Martín-Belloso, & Rojas-Graü, 2012).

Nanoemulsions also improve the biological activity of lipophilic compounds, such as EOs, due to their capacity of increasing solubility and dispensability in water-based foods, allowing to reach places where microorganisms proliferate (Donsì & Ferrari, 2016). Several studies have demonstrated that nanoemulsions containing EOs have greater antimicrobial activity than the bulk oil (Table 2), since

nano-sized droplets can interact more efficiently with the microbial cell membranes causing the microorganism death (Donsì & Ferrari, 2016). The nanometric particle size also allows minimizing the impact of EOs on food organoleptic properties and the risk of toxicity, by lowering the concentration needed for microbial inactivation. Recently, it has been reported that the minimal concentration of EO required to inhibit several pathogenic bacteria decreases using nanoemulsions (Moghimi, Aliahmadi, McClements, & Rafati, 2016). Bioactive nanoemulsions have greater bioavailability than conventional emulsions. Table 3 presents recent research works dealing with nanoemulsions as delivery systems of nutraceuticals. Lipophilic nutraceuticals encapsulated within nano-sized droplets are more soluble in the gastrointestinal fluids, so that higher concentrations of the bioactive compound can reach to the target site (Fathi et al., 2012). The lipid digestion occurs more rapid for nanoemulsions owing to the greater surface area exposed to the gastrointestinal fluids, which results in the release of encapsulated bioactive compounds in a greater extent.

3.1.3 Food applications

Nanoemulsions containing natural antimicrobials, such as EOs, have been used in fluid foods to increase their safety. For instance, Ghosh et al. (2014) incorporated nanoemulsions of eugenol in orange juice (0.3 % eugenol in the juice) and evaluated their inhibitory effect on native bacteria at 25ºC and 4ºC for 72 h. The microbial growth in orange juice significantly decreased after 6 h at both storage temperatures, but the inhibitory effect was greater at 4ºC after 72 h. In another work, Jo et al. (2015) incorporated nano-sized (<200 nm) cinnamaldehyde emulsions to watermelon juice at different concentrations of cinnamaldehyde-Tween, using a ratio of 1:3 (0.8 %, 2.4 % and 4 % w/w). The pathogenic bacteria growth lessened using the lowest antimicrobial concentration, compared with a control treatment.

The addition of polysaccharides with film-forming properties in the aqueous phase of nanoemulsions allow their application as edible films. Otoni et al. (2014) obtained antifungal films prepared from nanoemulsions of methylcellulose and clove or oregano oils to extend the shelf-life of packaged bread slides. They observed a clear reduction of the fungal growth on bread slides stored for 15 days. In

another study, edible films from nanoemulsions of cinnamaldehyde, pectin and papaya puree and were effective in inactivating pathogenic bacteria (Otoni, Moura, et al., 2014). Our group investigated the inhibitory effect against *E.coli* of alginate-based edible films formulated with nanoemulsions of three different essential oils. The antimicrobial effect was found to depend on the EO type, whereas the physicochemical properties of nanoemulsions significantly affected the physical and mechanical film properties. (Acevedo-Fani, Salvia-Trujillo, Rojas-Graü, & Martín-Belloso, 2015).

Nanoemulsions have been as well applied as edible coatings. Salvia-Trujillo et al., (2015b) applied conventional emulsions and nanoemulsions of lemongrass oil and sodium alginate as edible coatings on fresh-cut apples. Nanoemulsion-based coatings were more effective inactivating spoilage and pathogenic microorganisms than conventional emulsion-based coatings. The shelf-life of other fruits and vegetables including plums, berries, arugula, lettuce and green beans has been as well extended using nanoemulsion-based edible coatings of plant-based antimicrobials (Bhargava, Conti, da Rocha, & Zhang, 2015; Kim et al., 2013; Kim, Oh, Lee, Song, & Min, 2014; Sessa, Ferrari, & Donsì, 2015; Severino et al., 2015).

3.2 MULTILAYER EMULSIONS

Multilayer emulsions can be defined as oil-in-water systems containing droplets with at least two interfacial membranes composed by surfactants and biopolymers, which are created by the layer-by-layer assembly. Normally, an initial stable emulsion of nano-sized droplets is used to produce multilayer emulsions, and then several interfacial membranes are created by the alternative deposition of oppositely charged biopolymers; therefore, the multilayering process is driven mostly by electrostatic interactions, although other non-electrostatic forces may also play a role (Zeeb, Thongkaew, & Weiss, 2014). The presence of a multilayered membrane on droplets increases the physical stability of emulsions under certain environmental conditions, such as pH, ionic strength, heating, chilling or freeze-drying cycles, compared with one-layer emulsions (Aoki, Decker, & McClements, 2005; Fioramonti, Arzeni, Pilosof, Rubiolo, & Santiago, 2015). There are two principal mechanisms that promote the stability of multilayer emulsions: i) the steric stabilization caused when

biopolymers adsorb at the oil-water interface, which create a shield that protect particles from aggregation, and ii) the electrostatic stabilization driven by the strong Coulombic forces that provoke repulsion between droplets (Kuroiwa, Kobayashi, Chuah, Nakajima, & Ichikawa, 2015). Recently, multilayer emulsions have been proposed as potential delivery systems of active ingredients, owing to their outstanding physicochemical characteristics.

3.2.1 Factors affecting the formation and fabrication methods

The composition of multilayer emulsions and the preparation conditions significantly influences the formation of the interfacial membranes and the system stability. The biopolymer concentration in the aqueous phase has a significant impact in the multilayer emulsion formation. At very low concentrations, a biopolymer chain tends to adsorb to the interface of several droplets because there is insufficient material to coat all the interfacial area, causing then droplet aggregation by bridging flocculation. When the biopolymer concentration in the aqueous phase is high enough to saturate the entire droplets surface, then multilayer emulsions become physically stable. However, if the biopolymer concentration is too high there is an excess of non-adsorbed material remaining in the aqueous phase, which may cause droplets aggregation by depletion flocculation (Mun, Decker, & McClements, 2005). Another factor to consider is the droplets concentration and biopolymer-particle ratio. Mathematical analyses demonstrated that, as the particle concentration decreases the rate of flocculation also decreases because the biopolymer chains are adsorbed to the droplet surface more rapidly than the droplet collision (McClements, 2005). The preparation conditions such as pH, ionic strength or solvent quality have an important impact on the characteristics of the multilayered membranes, such as charge, thickness or porosity(Guzey & McClements, 2006). One of the most important factors affecting the formation and membranes characteristics is the pH of the system. Normally, most food-grade biopolymers behave as weak polyelectrolytes, which means that their charge density is strongly affected by the pH of the system. Either if they are adsorbed to droplets interface acting as surfactants or forming electrostatic complexes with another pre-adsorbed layer, the magnitude and sign of the electrical charge of such (macro) molecules would be changed by the

protonation or deprotonation of the functional groups that provides that charge (Fioramonti, Martinez, Pilosof, Rubiolo, & Santiago, 2015). Therefore, stronger or weaker interactions between oppositely charged species on the droplet surface would determine: i) the ability of forming electrostatic complexes or not, and ii): the characteristics of such multilayered membranes, such as the thickness, degree of porosity or electrical charge.

To produce multilayer emulsions, an oil-in-water "primary" emulsion is prepared, normally, by high-energy methods using an ionic surfactants or surface-active biopolymers with an electrical charge (Zeeb et al., 2014). In a further step, an oppositely charged biopolymer is incorporated into primary emulsions allowing their adsorption to the droplet interface, thus forming a new "secondary" emulsion. The process can be repeated several times to obtain emulsions with oil droplets coated by a desirable number of layers (Guzey & McClements, 2006). A schematic representation of the process of formation is presented in Fig.2. The most widely used method to fabricate multilayer emulsions is by adding exactly the amount of polyelectrolyte needed to saturate the droplets surface (Fioramonti, Martinez, et al., 2015; Pinheiro, Coimbra, & Vicente, 2016; Yang, Tian, Ho, & Huang, 2012). This method allows saving time in the process because the adsorption of polyelectrolytes occurs quickly, and there is no loss of polyelectrolytes because only the required amount is used. However, the correct balance of the components in the system is crucial to avoid bridging flocculation or depletion flocculation. Finding the polyelectrolyte concentration where a saturated layer is formed, either by empirical methods (monitoring the ζ-potential until stabilization) or by theoretical calculations, is a key factor to fabricate stable multilayer emulsions.

3.2.2 Advantages as delivery systems

These systems allow encapsulating both lipophilic and hydrophilic active compounds, locating them either in the lipid phase or in the interfacial coating. The high physical stability of multilayer emulsions under different environmental stresses is a major advantage when they are intended to be incorporated to food matrices. It is desirable that a delivery system maintains the functional properties of the encapsulated active compounds in presence of other food components. It has been described that

multilayer emulsions have the capacity of increase the chemical stability of encapsulated active compounds, since interfacial membranes protect lipophilic substances from degradation reactions (oxidation or light-induced) (Hou et al., 2010). The deposition of charged antioxidant molecules (e.g. polyphenols) at the oil-water interface of droplets can reduce oxidative degradation of β-carotene encapsulated in the lipid core (Liu, Wang, Sun, McClements, & Gao, 2016).

Furthermore, the thickness, porosity, composition or surface charge of the interfacial layers can be tuned in order to provide stimuli-responsive properties to emulsions, which is a great advantage for controlling the release of encapsulated active compounds. For instance, it is possible to slow down the lipophilic bioactive release by adding several interfacial membranes on droplets, and also to accelerate the release by changing the pH of the system. The pH might alter the conformation of the interfacial membranes, and in turn, can modulate the release rate of the active compound (Beicht, Zeeb, Gibis, Fischer, & Weiss, 2013). Lipid digestibility or bioaccessibility of nutraceuticals in the gastrointestinal tract (GIT) can be controlled using multilayer emulsions. The presence of protein-polysaccharide membranes on droplets may cause a delay or acceleration of the lipid digestion, which depend on the characteristics on the interfacial membranes and their physicochemical behavior in the different gastrointestinal fluids (Tokle, Lesmes, Decker, & McClements, 2012; Zeeb, Lopez-Pena, Weiss, & McClements, 2015). Within GIT, droplets stability may be influenced by the pH, ionic strength, enzymes, bile, among others components conforming the gastrointestinal fluids. Therefore, a tailored design of the interfacial membranes characteristics in multilayer emulsions can be a promising strategy to rationally develop efficient delivery systems for food ingredients. Most of the ongoing studies are exploring their behavior under gastrointestinal conditions, as they might be a promising alternative for enhancing the delivery of nutraceuticals. However, to the best of our knowledge, multilayer emulsions have not been yet explored as delivery systems of plant-based antimicrobials.

3.2.3 Food applications

The practical application of multilayer emulsions in food products is still scarce. However, there is an approach that suggests the use of multilayer emulsions containing bioactive lipids as a strategy to

enhance the fat quality of food products. Jo et al., (2015) prepared fish oil (high omega-3 fatty acids content) multilayer emulsions by the electrostatic deposition of Tween (primary), Tween-chitosan (secondary), and Tween-alginate-pectin (tertiary). Pork patties were enriched with fish oil encapsulated in primary, secondary and tertiary emulsions. The chemical stability of the fat contained in the product was assessed by the degree of lipid oxidation during storage, and it was found that the fish oil content in the pork patties had better chemical stability as the number of layers increased in multilayer emulsions.

3.3 NANOLAMINATES

Nanolaminates are thin films formed by two or more layers of food-grade materials alternatively deposited on a substrate through the layer-by-layer assembly technique. Typical layer thickness ranges from several Å to up to 100 nm, which depends on the adsorption conditions and material properties (Clark & Hammond, 1998). However, the final film thickness is governed by the number of layers deposited. The layer-by-layer assembly can be carried out *via* several chemical interactions, including electrostatic bonding, hydrogen bonding, hydrophobic interactions, charge-transfer interactions, covalent bonding, among others (Borges & Mano, 2014). So far, electrostatic interactions is the only mechanism explored to prepare nanolaminates in the food field (Flores-López, Cerqueira, de Rodríguez, & Vicente, 2016). The process starts with the adsorption of a charged specie (e.g. polysaccharides, proteins or nanoparticles) to a substrate that also has an electrical charge. The first adsorption step leads to a charge reversal on the surface that allows the adsorption of another oppositely charged specie. After each layer assembly, a washing step is required to remove the excess of unbound material, thus self-regulating the layer thickness. The sequential adsorption of oppositely charge building blocks is usually repeated to obtain nanolaminate structures. Numerous research works published in other science fields concerning the layer-by-layer technique point out that the mechanisms behind recharging and the driving forces that give rise to the nanolaminates buildup are still not fully understood (Borges & Mano, 2014). The intervention of other types of non-coulombic interactions acting synergistically with electrostatic attractions has been proposed (Schoeler, Sharpe,

Hatton, & Caruso, 2004). Lately, some studies suggest that nanolaminates prepared from polysaccharides may exhibit exceptional physicochemical characteristics, such as gas barrier properties, water vapor resistance or different wetting properties and surface charge, compared to conventional materials (Li, Biagioni, Finazzi, Tavazzi, & Piergiovanni, 2013; Pinheiro et al., 2012). Additionally, a high swelling capacity has been observed in polysaccharide-based nanolaminates (Crouzier, Boudou, & Picart, 2010), which may have important implications in the diffusion of the other molecules (e.g. small active molecules) inside the structure.

3.3.1 Factors affecting the formation and fabrication methods

There are several experimental parameters that affect the formation and physicochemical properties of nanolaminates, such as the pH, ionic strength, concentration and temperature of the solutions containing the building blocks (e.g. polysaccharides or proteins), the molecular weight or charge density of the building blocks, number of layers deposited, the terminal layer, adsorption and washing time, and film drying procedure (Klitzing & Klitzing, 2006). The pH and ionic strength of the adsorbing solutions are among the most important factors affecting the nanolaminates assembly. The pH has a profound effect on the conformation and charge density of polysaccharides in solution and hence, on their kinetics of adsorption on the substrate. This directly changes the nanolaminate growth and, aspects such as film thickness, surface charge, wettability or roughness will be affected. For instance, the conformation of ionic biopolymers may vary from well-extended to globular depending on the solution pH, therefore, the amount of mass adsorbed per layer will be different in each condition (Acevedo-Fani, Salvia-Trujillo, Soliva-Fortuny, & Martín-Belloso, 2015). The ionic strength also affects the formation of nanolaminates. The concentration of salt in the solution influences the conformation of biopolymers due to the screening of the charges along the chains, resulting in coiled structures. Thereby, the nanolaminate thickness can be increased if the ionic concentration is increased (Klitzing & Klitzing, 2006).

There are three main ways to prepare nanolaminates by the LbL technique: i) dipping, ii) spraying and iii) spin coating (Benkirane-Jessel et al., 2011). The dipping method is most commonly used to form

nanolaminates and can be carried out manually by simply submerging the charged substrate on an adsorbing solution, or mechanically, using a device that controls the number of immersion steps and the duration of adsorption and washing steps. In this case, adsorption steps can last between 1 min and 1 h (Bertrand, Jonas, Laschewsky, & Legras, 2000). The layer-by-layer deposition by spraying and spin-coaters has also been confirmed but it has not been used in the food field. Such methods present the advantages of using a small amount of material to coat a surface and the velocity of the adsorption process, which can occur more rapidly in comparison to the dipping approach (Aoki et al., 2014).

3.3.2 Advantages as delivery systems

One of the most promising advantages of nanolaminates is their ability of entrap a payload of active compounds within the film structure and then release it in response to external stimulus such as pH, ionic strength, light, temperature, etc (Keeney et al., 2015). So far, nanolaminates have mostly been explored as food-grade coatings on packaging materials and some commodities. Moreover, the incorporation of active substances within nanolaminates is also possible, either by changing the nature of the building blocks adsorbed in each layer or by the loading of small active molecules. Therefore, these nanolaminates can work as carriers of active ingredients with antimicrobial or health-promoting properties. Nanolaminates can be designed to control the release of encapsulated substances under specific triggers, enhancing their targeted delivery. As far as we are concerned, this topic has been scarcely investigated. Therefore, there is a lack of information that opens the possibility to a future research trend, so that the potential advantages of nanolaminates for active ingredients delivery can be explored in a greater extent, being a promising alternative to improve safety, quality and functionality of foodstuffs. The versatility of the layer-by-layer assembly could allow the design of countless types of food-grade nanolaminates with tuned physicochemical and functional properties, either by modulating the layer composition or assembly parameters, which would have a positive impact in food preservation or fortification.

3.3.3 Food applications

The application of nanolaminates in foods is in the early stage. Nanolaminates have been formed on food surfaces or conventional packaging materials. A schematic representation of the procedure to create nanolaminate structures onto solid foods is shown in Fig. 3. The formation and characterization of nanolaminates on planar materials is a crucial preliminary step to their application as edible coatings. This is considered as an 'in vitro' study that allows confirming the actual formation and properties of nanolaminates. The typical microstructure of a nanolaminate formed onto a polyethylene terephthalate sheet is presented in Fig. 4. For instance, Pinheiro et al., (2012) reported the structural and transport properties of κ-carragenan and chitosan nanolaminates created on a polyethylene terephthalate film (PET). The layer-by-layer deposition of both oppositely charged polysaccharides forming nanolaminates was confirmed, and their influence on wetting and gas barrier properties of the substrate were also assessed. Our group studied the influence of the electrical charge of alginate and chitosan solutions on the buildup and physicochemical properties of the resulting nanolaminates created on PET and quartz slides (Acevedo-Fani, Salvia-Trujillo, Soliva-Fortuny, et al., 2015). As nanolaminates may serve as reservoirs of active molecules, some authors have demonstrated their ability to hold antimicrobial molecules and nanoparticles within the film structure. In fact, nanofibrous mats with antimicrobial activity were successfully obtained by applying a nanolaminate coating of alginate (negative) and a lysozyme-chitosan-rectorite complex (positive) on the surface. Coated mats exhibited higher antimicrobial activity when the nanolaminate was made of the lysozyme-chitosan-rectorite complex and their use on pork preservation extended the shelf life for about 3 days (Huang et al., 2012). In another case, it was possible to fabricate nanolaminate films of PET using nanocapsules or emulsions containing carvacrol as building blocks. This improved the antifungal properties of the packaging material (Fabra et al., 2016). The use of nanolaminates as edible coatings has also been demonstrated. For instance, chitosan, alginate, κ-carragenan or lysozyme were used to produce nanolaminates that were able to preserve the quality and safety of fresh-cut and whole pears and mangoes (de S. Medeiros, Pinheiro, Carneiro-da-Cunha, & Vicente, 2012; Medeiros, Pinheiro,

Teixeira, Vicente, & Carneiro-da-Cunha, 2012; Souza et al., 2015). The same approach has been used for extending the shelf-life of cheese (de S. Medeiros et al., 2014).

4 TOXICOLOGICAL ASPECTS

Nowadays, the number of nanotech patents and nano-products being released to the food market is increasing, but there is also a great concern about the potential toxicological effects related to their intake through foodstuffs. It is noteworthy that the potential advantages of nanostructured delivery systems arising from the manipulation of materials in the nanometric scale (e.g. greater reactivity, higher bioavailability, enhanced cellular transport) may also have an important impact on their toxicity in the human body. For example, some nutraceuticals are toxic at high concentrations and their intake is recommended at certain doses. Nutraceuticals encapsulated within nanostructured delivery systems may cause toxicity due to their greater bioavailability, thereby, increasing the bioactive concentration in the target site. Essential (EOs) oils may also have some toxic effects associated to their potential ability to interact with the human cellular membranes in the same way that EOs acts on microbial membranes. Therefore, if EOs are encapsulated in nanostructured systems their increased reactivity may represent a latent issue in terms of safety. Moreover, some nanoparticulates may not be digested in the GIT, being able to pass across the epithelium cells and increasing the exposure to biological tissues, which could lead to unpredictable interactions or bioaccumulation in some organs. However, if nanomaterials are transformed into bigger structures in the food matrix before ingestion or they are completely degraded and solubilized in the GIT, then the toxicity risk may be insignificant. Therefore, the evaluation of toxicological profiles and potential risks associated with the use of nanomaterials in foods is required. The toxicity of nanomaterials strongly depends on their physicochemical properties including chemical composition, size, shape, solubility, surface charge, surface reactivity, among others, as well as their behavior within the GIT and final fate in the human body after adsorption. In this regard, risk assessment must be done considering such characteristics and following an approach based in the case-by-case study.

5 CONCLUDING REMARKS

There is an evident interest in the design and production of food-grade nanostructures able to encapsulate, protect and enhance functionality of some types of active ingredients. Plant-derived antimicrobials and nutraceuticals are potential natural additives that can be incorporated within foodstuffs, representing a promising strategy to satisfy the current consumer's claims. The proper formation of nanostructured delivery systems have to be carefully controlled in order to reach stable systems. There are a number of factors that intervene differently in each type of delivery system. In the case of nanostructured emulsions, the surfactant type, surfactant concentration, viscosity of the aqueous media, the biopolymer concentration, pH, ionic strength are among the most important factors affecting the formation and stability of droplets. The assembly of nanolaminates is highly influenced by the conditions of biopolymer solutions (e.g. pH, ionic strength), but also by different experimental parameters (e.g. adsorption and washing times, number of layers, terminal layer, drying procedure). Several research works has indicated that nanostructured emulsions and nanolaminates are able to encapsulate both plant-derived antimicrobials and nutraceuticals, providing enhanced physicochemical stability and functional properties. Their ability to modulate the biological fate of bioactive ingredients within GIT, and to control their release under certain conditions seems to be a promising advantage for the design of functional foods. Applications in food models are still incipient. Up to date, some research works conducted suggest that both nanostructured emulsions and nanolaminates have the potential to improve food preservation and food fortification, but there are still unknown aspects that required further research. Another latent concern is the potential toxicity effect of food nanostructures in the human body. The novel properties arising from manipulation of materials and active ingredients at the nanoscale may have important consequences in the toxicity after consumption, which should be considered in a case-by-case basis. Therefore, future investigations have to be directed to a more comprehensive approach of all the factors implied in the use of nanostructured delivery systems in foodstuffs, starting from their physicochemical stability and functional performance, stability in food

matrices during storage, behavior during digestion, potential toxicological effects, until the economic analysis of the costs implicit in the industrial scale-up.

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720 **Table 1.** Plant-derived antimicrobials and nutraceuticals that can be encapsulated within

721 nanostructured emulsions and nanolaminates.

Compound	Types	Bioactivities
Plant-based antimicrobials		
EOs	Thyme, oregano, sage, lemongrass, cinnamon, rosemary, marjoram, clove	Antimicrobial, antioxidant
Active compounds of EOs	Carvacrol, cinnamaldehyde, thymol,eugenol, citral	Antimicrobial, antioxidant
Nutraceuticals		
Micronutrients	Lipophilic and hydrophilic vitamins (vit. A, vit. D, folic acid). Minerals (zinc, magnesium)	Co-enzymes for metabolic process, antioxidants, modulation of gene transcription
Phytochemicals	*Carotenoids* (carotene, lycopene), *terpenoids and isoterpenoids* (resveratrol), *phenolics* (isoflavones, anthocyanins)	Antoxidants, anti-inflammatory, anti-hyperlipidemic, chemopreventive activity
Fatty acids	omega-3 fatty acids, omega-6 fatty acids	Brain development, cardio-protection, anti-inflammatory action
Bioactive peptides and proteins	Val-Tyr-Pro, lactoglobulin and lactoferrin	Cardio-protection, anti-inflammatory action

722 Adapted from (Gleeson et al., 2016) and (Bassolé & Juliani, 2012)

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724 **Table 2**. Recent studies regarding the use of nanoemulsions and nanolaminates for plant-derived antimicrobials delivery.

	Active compound	Technique	Materials	Particle size / film thickness	Outcomes	References
Nanoemulsions	Sage oil	Sonication	Emulsifiers: Tween 80 and Span 80	≈200 nm	Greater antimicrobial activity than bulk oil against pathogenic bacteria	(Moghimi et al., 2016)
	Anise oil	HPH	Disperse phase: MCT oil Emulsifier: Soy lecithin	<276 nm	Better long-term stability and antimicrobial activity than bulk oil	(Topuz et al., 2016)
	Thyme oil	HPH	Disperse phase: corn oil Emulsifiers: lauric arginate and Tween 80	<250 nm	Enhanced antimicrobial activity against spoilage yeast combining lauric arginate and thyme oil	(Chang, McLandsborough, & McClements, 2015)
	Lemongrass, clove, tea tree, thyme, geranium, marjoram, palmarosa, rosewood, sage and mint	MF	Emulsifier: Tween 80 Texturizing agent: sodium alginate	<20 nm	Nanoemulsions exhibited faster inactivation of E.coli than cconventional emulsions	(Salvia-Trujillo, Rojas-Graü, Soliva-Fortuny, & Martín-Belloso, 2015a)
	Carvacrol	Spontaneous emulsification	Disperse phase: MCT oil Emulsifiers: Tween 20, 40, 60, 80 and 85	≈55 nm	The antimicrobial activity against spoilage yeast increased with oil concentration. Nanoemulsion stability was better at low oil concentrations	(Chang, McLandsborough, & McClements, 2013)
	Peppermint	HPH	Disperse phase: MCT oil Emulsifier: modified starch	<200 nm	Greater antimicrobial activity against pathogenic bacteria than bulk oil. High long-term stability	(Liang et al., 2012)
Nanolaminates	Carvacrol	Layer-by-layer	Bulding blocks: alginate, zein-carvacrol nanocapsules, chitosan and chitosan-carvacrol emulsions Substrate: PET sheet	Not reported	Water and oxygen transmission rate decreased compared with net alginate or chitosan films. Nanolaminates exhibit antifungal activity against Alternaria ssp.	(Fabra et al., 2016)

725 HPH: high pressure homogenization; MF: microfluidization

726 **Table 3**. Recent studies regarding the use of nanostructured emulsions for lipophilic nutraceutical delivery.

	Active compound	Technique	Materials	Particle size	Outcomes	References
Nanoemulsions	Green tea catechins	HPH	Disperse phase: Sunflower oil Emulsifier: soy protein	240-270 nm	High physical and chemical stability in cold storage Cathechins nanoemulsions exhibited greater bioaccessibility and permeability through caco-2 cell monolayers than non-encapsulated cathechins	(Bhushani, Karthik, & Anandharamakrishnan, 2016)
	Vitamin E	MF	Disperse phase: Sunflower oil Emulsifier: saponins	≈ 300 nm	High physical stability during storage at different temperatures Nanoemulsions exhibited higher vitamin E bioavailability than conventional emulsions, assessed by *in vivo* studies	(Parthasarathi, Muthukumar, & Anandharamakrishnan, 2016)
	Vitamin D_3	HPH	Disperse phase: LCT* oils (corn and fish oils), MCT* oil, indigestible oils (mineral and orange oils) Emulsifier: Quillaja saponin	≈ 190 nm	Vitamin D bioaccessibility increased in LCT nanoemulsions, compared to MCT nanoemulsions. Undigested oil nanoemulsions presented greater vitamin bioaccessibility than MCT nanoemulsions	(Ozturk, Argin, Ozilgen, & McClements, 2015)
	Resveratrol	HPH	Disperse phase: peanut oil Emulsifiers: combinations of lecithins, sugar esters, Tween 20 and glycerol monooleate	130 – 240 nm	Transport though caco-2 cells monolayers was enhanced in resveratrol nanoemulsions with the smallest droplet size. The resveratrol metabolization by caco-2 cells and the *in vitro* release was lower in nanoemulsions	(Sessa et al., 2014)
	β-carotene	MF	Disperse phase: corn oil Emulsifier: Tween 20	23 – 0.2 um	The initial droplet size affected β-carotene bioaccessibility. It increased by decreasing droplet size of emulsions.	(Salvia-Trujillo, Qian, Martín-Belloso, & McClements, 2013)
Multilayer emulsions	Curcumin	HPH and LbL	Disperse phase: corn oil Emulsifier: lactoferrin Coatings composition: lactoferrin and	> 150 nm	Interfacial membranes allowed controlling lipid digestion and curcumin release in different stages of the small intestine	(Pinheiro et al., 2016)

			lactoferrin/alginate			
	β-carotene	HPH and LbL	Disperse phase: MCT* oil Emulsifier: lactoferrin and lactoferrin-polyphenol conjugates Coatings composition: lactoferrin, lactoferrin-polyphenols, lactoferrin/lactoferrin-polyphenols	≈ 300 – 1300 nm	The presence of polyphenol in the interfacial layers helped to improve the physical and chemical stability of β-carotene encapsulated. The type of polyphenol affected the stability profiles of emulsions.	(Liu et al., 2016)
	Carotenoids	MF and LbL	Disperse phase: MCT oil Emulsifier: soy protein isolate (SPI) Coatings composition: SPI, SPI/alginate, SPI/chitosan	≈ 280 nm	Lipid digestion decreased when the outer layer on droplets was chitosan. Carotenoids bioaccessibility increased in SPI/alginate emulsions.	(Zhang et al., 2015)
	Lutein	HPH and LbL	Disperse phase: MCT* oil Emulsifiers: WPI*, DTAB and fish gelatin (FG) Coatings: WPI/sugar beet pectin, DTAB/sugar beet pectin and FG/sugar beet pectin Crosslinked coatings with lacasse	< 200 nm	Multilayer emulsions significantly diminished lutein release in comparison with one-layer emulsions. The release profiles of crosslinked emulsions were similar to multilayer emulsions	(Beicht et al., 2013)

727 *MCT: medium chain triglyceride oils; LCT: long chain triglyceride oils; WPI: whey protein isolate; LbL: layer-by-layer deposition; HPH: high pressure homogenization; MF: microfluidization

Fig. 1. Food nanostructured systems for encapsulating active ingredients with potential applications in beverages or edible coatings.

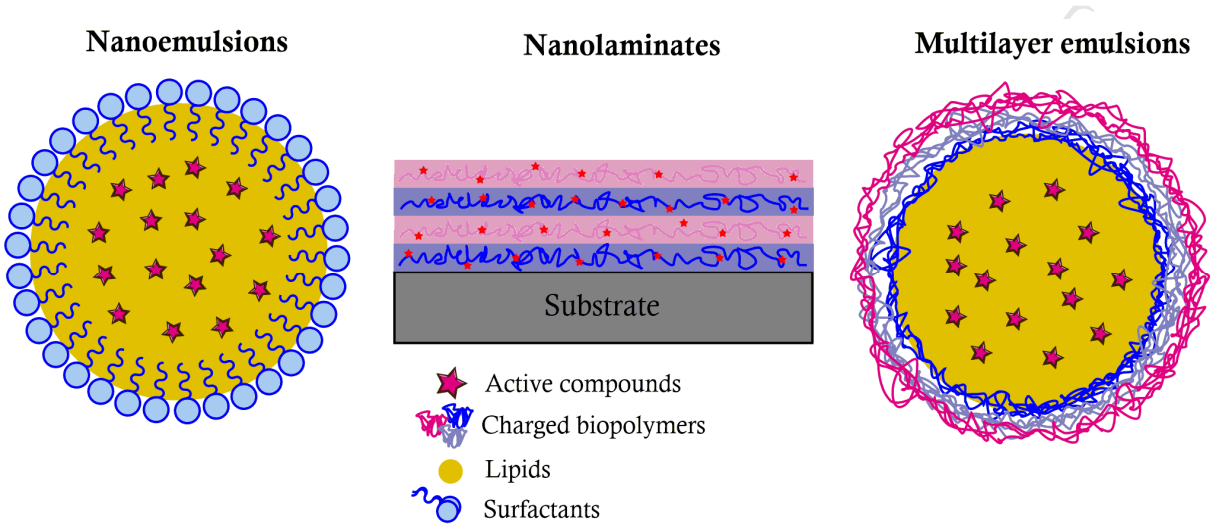


Fig. 2. Schematic representation of the fabrication process of multilayer emulsions.

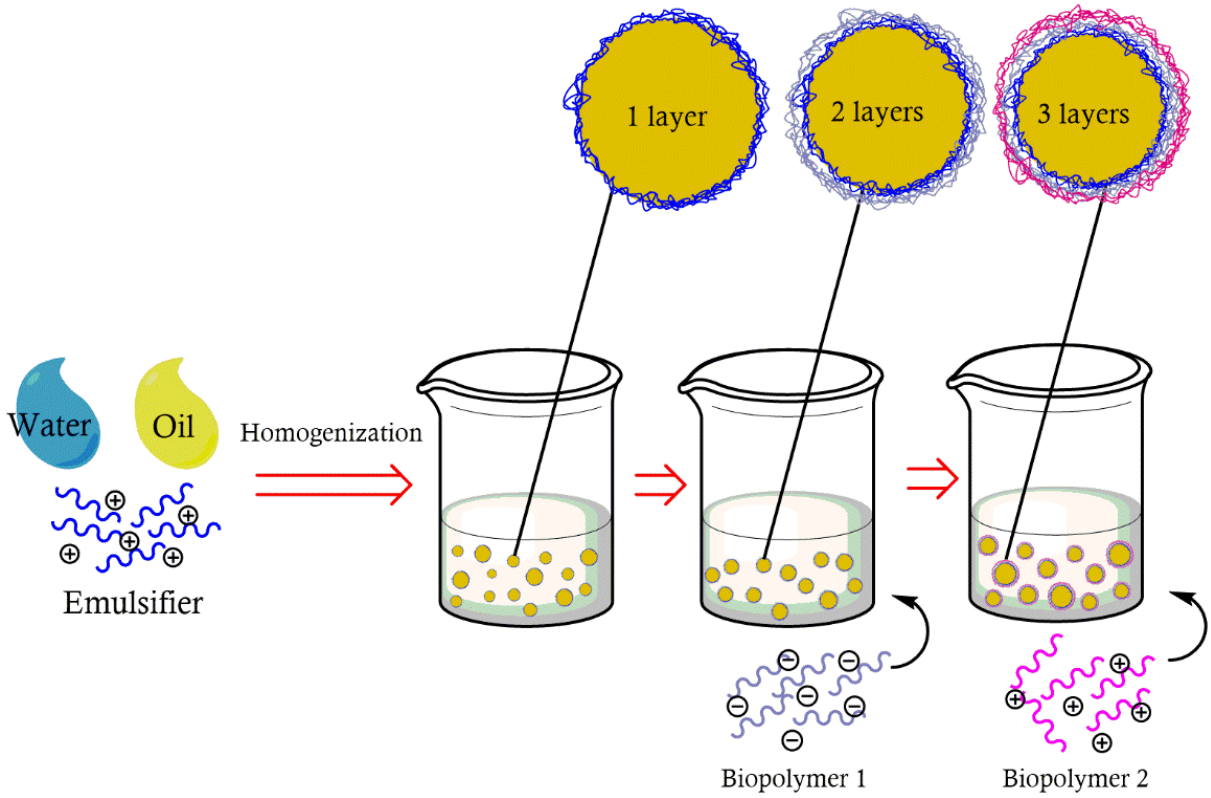


Fig. 3. Schematic representation of the layer-by-layer assembly on food surfaces.

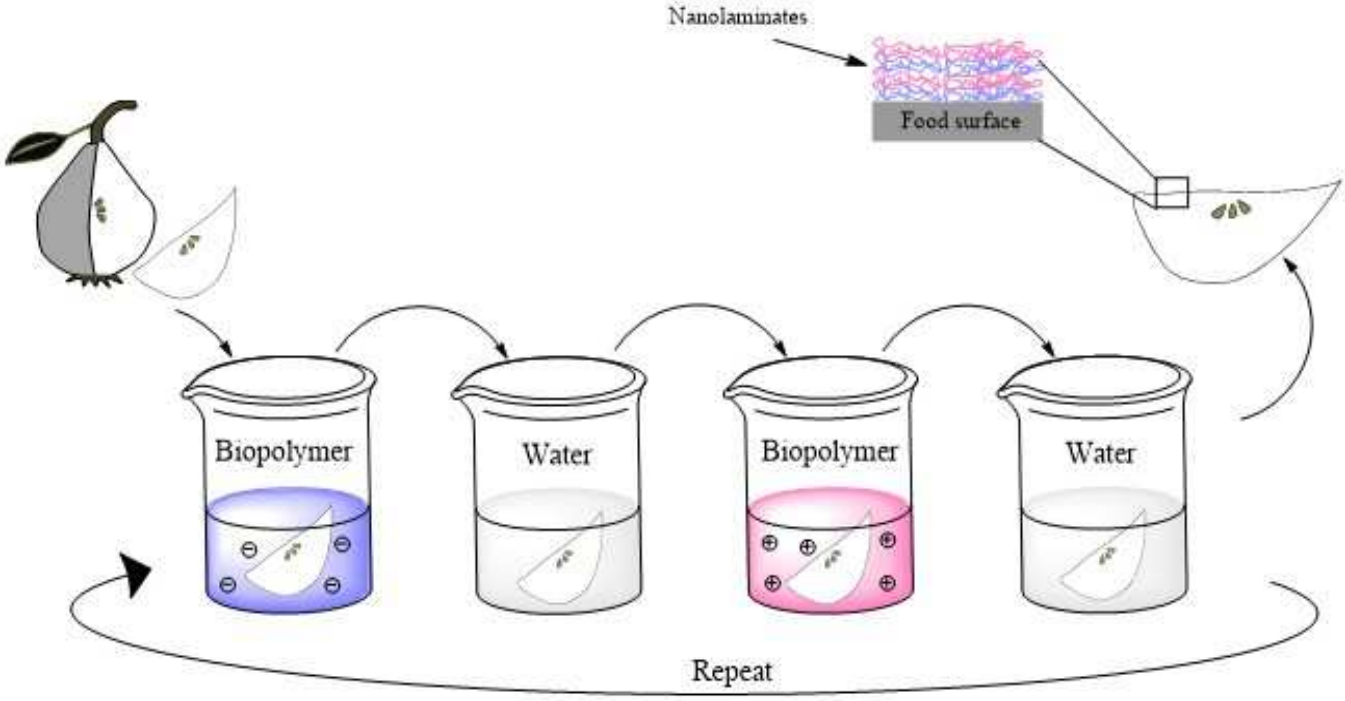
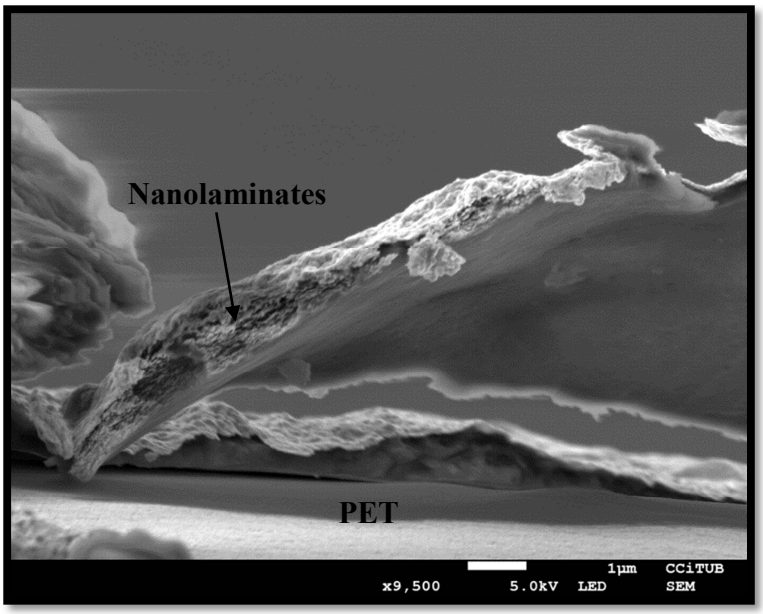


Fig. 4. SEM micrograph of alginate/chitosan nanolaminates formed on polyethylene terephthalate sheet. (Acevedo-Fani, Salvia-Trujillo, Soliva-Fortuny, et al., 2015)



Highlights

- There is a trend in using plant-based antimicrobials and nutraceuticals for improving food properties, but their intrinsic characteristics and high instability are limiting factors.
- Nanostructured emulsions and nanolaminates are able to encapsulate, protect and enhance the delivery of such substances.
- The properties of nanostructured delivery systems can be controlled, manipulating the experimental parameters during their formation.
- Recent studies indicates potential applications in beverages, solid foods or edible films and coatings.
- The potential toxicity of nanostructured systems represent a major concern that require deeper research.